



THROMBOCYTOPENIA AMONGST PATIENTS: COMPARISON OF MALARIA VERSUS NON-MALARIA PATIENTS

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ABSTRACT

Background: Platelet count forms part of routine complete blood count in medical laboratories. With advancement in technology, automated machines with their own reference values are now used by most laboratories in the estimation platelets and other blood parameters. The limitations with the automated machines are that the risk misdiagnosing patients with abnormal platelet count are high since in often times, the reference values provided by the reagent manufacturers do not reflect the platelet count of the desired population. The normal range of blood platelet is $150-400 \times 10^3/\mu\text{L}$ hence platelet counts below $150 \times 10^3/\mu\text{L}$ and above $400 \times 10^3/\mu\text{L}$ are described as abnormal. Malaria infection has been reported as one of the causes of low platelet count in patients. **Objective:** To corroborate and assess the platelet levels in malaria and non-malaria patients to justify the need for laboratories to generate their own reference values. **Methods:** This present study was conducted on 599 patients of which 169 tested positive for malaria and 430 tested negative for malaria in the Ashanti-Bekwai Municipal Hospital from November 2016 to May 2017. Blood sample was collected, thick and thin film slide were prepared and examined under microscope and automated machine (Sysmex KX-21N) was used to determine platelet level. **Results:** Among the study population, 169 (28.21 %) were diagnosed of malaria whilst 430 (71.79 %) had no malaria infection. Also, 109 (25.35 %) were recorded to have thrombocytopenia amongst the malaria negative group whilst 139 (82.85 %) were recorded to have thrombocytopenia amongst the malaria positive group. **Conclusion:** From this present study, it was revealed that thrombocytopenia was present in both malaria and non-malaria patients.

KEY WORDS

Malaria, Thrombocytopenia, Pseudothrombocytopenia, Platelet

INTRODUCTION

Platelets belong to a group of blood cells which are produced in the bone marrow by the fragmentation of

the cytoplasm of megakaryocytes. The production of platelets is mainly regulated by thrombopoietin which is produced constitutively by the liver and kidneys [1].

Following the production of platelets, they last for 7-10 days. Platelet count forms part of routine complete blood count in medical laboratories. With the advancement in technology, automated machines are now used in estimating the various blood parameters. Most laboratories rely solely on reference values provided by reagent manufacturers which may not necessarily reflect the normal range of the desired population. Failure to recognize artificial or spurious platelet count may result in incorrect diagnosis and treatment. The normal range of blood platelet is $150-400 \times 10^3/\mu\text{L}$ hence platelet counts below $150 \times 10^3/\mu\text{L}$ and above $400 \times 10^3/\mu\text{L}$ is described as abnormal platelet levels [1].

Thrombocytopenia occurs when blood platelet levels fall below $150 \times 10^3/\mu\text{L}$. This abnormality could be associated to either a decrease in platelet production, an increase in platelet destruction and a significant change or diversion in platelet distribution [2] Pseudothrombocytopenia also occurs especially with automated complete blood count machines in which the platelet count obtained does not correlate with the actual platelet level in the body of the patient [3].

Malaria is classified as one of the major health problems in Africa and it is associated with high morbidity and mortality [4]. Amongst the various species of the malaria parasite, *Plasmodium falciparum* is the commonest in Ghana [5]. Platelets play a critical role in the pathogenesis of malarial infections. Platelets enhance the sequestration of infected red blood cells within the cerebral vasculature and they also have well-established roles in innate protection against microbial infections. However, malaria infection has been reported as one of the causes of low platelet levels in malaria patients [7].

This study therefore sought to corroborate and assess thrombocytopenia in malaria and non-malaria patients and to also justify the need for laboratories to generate their own reference values and develop means of confirming artifactual or Pseudothrombocytopenia.

METHODOLOGY

This present study was conducted on 599 cases of which 169 tested positive for malaria and 430 tested negative for malaria in the Ashanti-Bekwai Municipal Hospital from November 2016 to May 2017.

Data Collection

Case report book in the facility's laboratory which contained the Patient's Name, Pathology Number, age, sex, address, clinical diagnosis and laboratory results was used for this study. Following the routine practices in this facility, the patient's blood sample was collected at the phlebotomy section into an EDTA tube and a thick and thin film slides were prepared for examination under a microscope for the presence malaria parasites. The blood sample collected into the EDTA tube was used to determine the platelet count using an automated complete blood count machine (Sysmex KX-21N). Laboratory results for Patients with no history of haemorrhagic disorders, no history of alcohol use, no echymosis, no recent melanoma, metrorrhagia, haemorrhagia diathesis, bruises, lymphadenopathy, unexplained weight loss on physical examination were included in the study while pregnant women, patients with liver disease, bleeding disorders were excluded from this study. Various platelet count were compared. $150-400 \times 10^3/\mu\text{L}$ was defined as the normal platelet level, less than $150 \times 10^3/\mu\text{L}$ was defined as low (Thrombocytopenia) with $50-150 \times 10^3/\mu\text{L}$, $20-49 \times 10^3/\mu\text{L}$ and less than $20 \times 10^3/\mu\text{L}$ respectively defined as mild, moderate and severe thrombocytopenia in this present study.

RESULTS

Out of the 599 cases that were analysed, 169 constituting 28.21 % tested positive for malaria while the remaining 430 constituting 71.79 % tested negative for malaria. For the malaria positive cases, 30 (17.75 %) had normal platelet count with mean value of $279.3 \pm 9.17 \times 10^3/\mu\text{L}$. Mild thrombocytopenia; mean platelet count of $102.3 \pm 2.78 \times 10^3/\mu\text{L}$ was recorded in 86 (50.89 %) of the malaria positive patients. 43 (25.44 %) of the malaria positive cases also had a moderate thrombocytopenia with a mean value of $34.54 \pm 1.51 \times 10^3/\mu\text{L}$. Severe thrombocytopenia; mean platelet count of $17.40 \pm 0.91 \times 10^3/\mu\text{L}$ was recorded among 10 (5.92 %) patients of the malaria positive group. In all, 139 (82.85 %) of patients belonging to the positive group had thrombocytopenia with the mean platelet count of $76.55 \pm 3.46 \times 10^3/\mu\text{L}$ whilst 30 (17.75%) of patients in the same category had normal platelet count. Among the malaria negative group, 321 (74.65 %) of them had normal platelet count with mean value of $245.4 \pm 3.66 \times 10^3/\mu\text{L}$ whilst 109 (25.35 %) had

thrombocytopenia. 97 (22.56 %), 9 (2.09 %) and 3 (0.70 %) had mild, moderate and severe thrombocytopenia respectively.

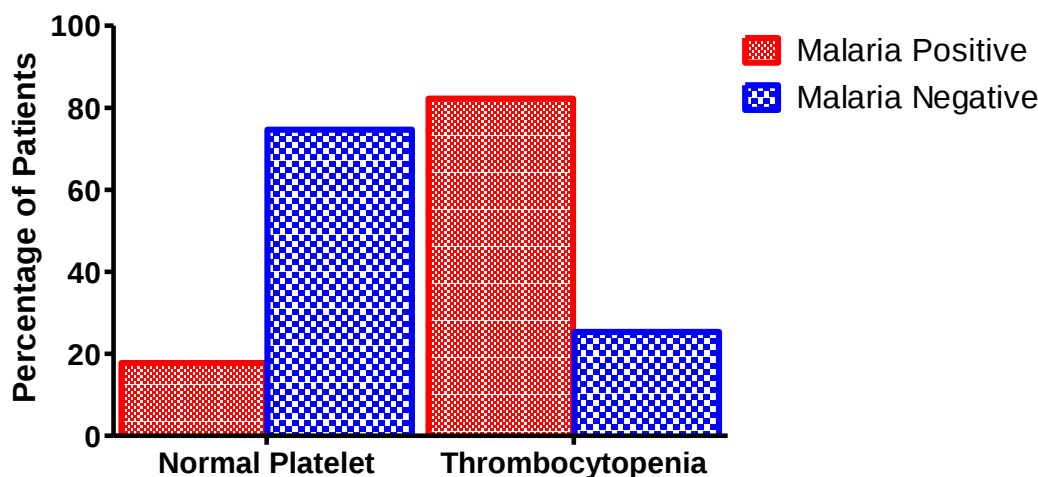
Statistical analysis.

Graphpad® Prism for Windows Version 7.0 (Graphpad Software, San Diego, CA, USA, 2016) was used for all statistical analysis. $P < 0.05$ was considered statistically significant for all tests.

Table 1: Depicts the platelet level of all patients (N=599) in this present study with their respective percentages and Mean $\pm$ SEM

Platelet Count	No. of Patients	Percentage	Mean $\pm$ SEM	Range ($\times 10^3/\mu\text{L}$)
Malaria Positive(n=169)				
Normal	30	17.75	279.3 $\pm$ 9.17	150-400
Mild	86	50.89	102.3 $\pm$ 2.78	50-150
Moderate	43	25.44	34.54 $\pm$ 1.51	20-49
Severe	10	5.92	17.40 $\pm$ 0.91	<20
Thrombocytopenia	139	82.85	76.55 $\pm$ 3.46	<20-150
Malaria Negative(n=430)				
Normal	321	74.65	245.4 $\pm$ 3.66	150-400
Mild	97	22.56	106.5 $\pm$ 2.846	50-150
Moderate	9	2.09	34.11 $\pm$ 2.39	20-49
Severe	3	0.70	14.67 $\pm$ 2.33	<20
Thrombocytopenia	109	25.35	97.98 $\pm$ 3.45	<20-150

Figure 1.0 Graphical representation of thrombocytopenia among malaria positive and malaria negative patients considered in this present study.



DISCUSSION

This study revealed that 139 (82.85 %) patients had thrombocytopenia with 86 (50.89 %) of them having mild thrombocytopenia, 43 (25.44 %) moderate thrombocytopenia and 10 (5.92 %) had severe thrombocytopenia whilst 30 (17.75 %) had normal platelet count. Two separate studies carried out in Pakistan showed that overall, 87.27 % and 85.5 % of malaria patients have low platelet count [6, 7]. Malaria is usually associated with various degrees of

thrombocytopenia [8]. Mechanisms such as immune mediated destruction of circulating thrombocytes, splenic pooling and reduced platelet lifespan due to malaria infection have been proposed [6]. Therefore, there is vindication by similar works that malaria is associated with different degrees of thrombocytopenia as revealed by this present study.

However, 25.35% patients with no malaria infection had thrombocytopenia. Due to the exclusion criteria invoked in this study, this thrombocytopenia could be

artificial or Pseudothrombocytopenia since there was no confirmation of the thrombocytopenia by other laboratory methods like changing the anticoagulant, using microscopy to detect platelet clumping. Pseudothrombocytopenia is a common laboratory phenomenon in hospitals [9,10]. It may be EDTA-dependent where the presence of autoantibodies directed against glycoprotein IIb/IIIa on the platelet membrane results in the clumping of platelets [11]. This can be identified by the presence of platelet clumps in peripheral smear of EDTA anticoagulant blood. The clumping can be prevented by changing the anticoagulant to Heparin or sodium citrate [12], collecting and examining blood at 37°C [13], supplementation of aminoglycosides to anticoagulants [14] and the addition of kanamycin [11]. Also, overreliance on reference values provided by reagent manufacturers which may not necessarily correlate with the normal range in the desired population or the vicinity of the laboratory may also result in false platelet counts. Wang *et al* 2004 recommended that laboratories using automated machines should develop their own reference values [15]. Thrombocytopenia could have been reduced in the non-malaria patients if the laboratory had generated their own reference values to be in sync with the laboratory's vicinity.

CONCLUSION

From this present study, it was revealed that thrombocytopenia was present in both malaria and non-malaria patients in Ashanti Bekwai Municipal Hospital. However, these patients need no platelet transfusion since this condition is can be described as self-limiting.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES

- [1] Italiano J.E. and Shivdasani RA (2003) Megakaryocytes and beyond: the birth of platelets. *J. Thrombo Haemost* (1):1174-1182.
- [2] Mehmet Ali Erkurta, Emin Kayaa, İlhami Berbera, Mustafa Koroglu, İrfan Kukua(2012) Thrombocytopenia in Adults: Review Article. *J Hematol* 1(2-3):44-5.
- [3] Geok C T, Mellisa S, Gretchen D, Maria N and Samir B K (2016). Pseudothrombocytopenia due to platelet clumping: a case report and brief review of literature. Case report in Haematology
- [4] Jadhav U.M, Patkar V.S, Kadam N.N (2004). Thrombocytopenia in Malaria, Correlation with Type and Severity of Malaria. *J Assoc Physicians India* 52:615-8
- [5] Wickramasinghe NS, Abdalla SH (2000). Blood and bone marrow changes in malaria. *Clinical Haematology* 13:277-99.
- [6] Hassan A, Ahmed A, Ahmed S, Abdulla M, Hassan MH, Rania M (2009). Thrombocytopenia in adults with acute malaria. *Rawal Med J* 34:170-3.
- [7] Mumtaz Ali Shaikh, Shakeel Ahmed, Inayat Ullah Diju, Dur-E-Yakta(2011) Platelet count in malaria patients. *J Ayub Med Coll Abbottabad* 2011;23(1).
- [8] Sheraz J, Fazal R, Muhammad U, Sameena Z (2008) Malaria can lead to Thrombocytopenia. *Rawal Med J* 33:183-5
- [9] Gschwandtner ME, Siostrzonek P, Bodinger C, Neunteufl T, Zauner C, Heinz G, Maurer G, Panzer S (1997). Documented sudden onset of Pseudothrombocytopenia. *Ann Hematol* 74:283-285.
- [10] Fang C, Chien Y, Yang I, Lu W and Lin M (2005). EDTA-Dependent Thrombocytopenia. *Journal of surgery* 48(3): 107-109.
- [11] Hae Lyun Ahn, Young Jo, Young Suk Choi, Jung Yeon Lee, Hae Woon Lee, Seong Ryul Kim, Joon Sim (2002). EDTA-Dependent Pseudothrombocytopenia confirmed by supplementation of kanamycin; A Case report. *Korean J Intern Med* 17(1): 65-68.
- [12] Lombarts AJPF, De Kieviet W (1988). Recognition and Prevention of Pseudothrombocytopenia and Concomitant Pseudoleukocytosis. *American Journal of clinical pathology* 89: 634-639.
- [13] Bizzaro N (1995). EDTA-dependent pseudos thrombocytopenia: a clinical and epidemiological study of 112 cases, with 10year follow-up. *Am J Hematol*. 50(2): 103-109.
- [14] Fiorin F, Steffan A, Pradella P, Bizzaro N, Potenza R, De Angelis V (1998). IgG platelet antibodies in EDTA-dependent Pseudothrombocytopenia bind to platelet membrane glycoprotein. *Am J Clin Pathol* 110: 178-183
- [15]. Wang C, Caitton D H, Demers LM *et al* (2004). Measurement of total serum testosterone in adult men: Comparison of current laboratory methods versus liquid



chromatography-tandem mass spectroscopy. Journal of clinical endocrinology 89:534-548.

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